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The Effects of Prenatal Exposure of Valproic Acid on Cranial Nerve Nuclei: A Rat Model for an Autistic Phenotype

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I. Introduction

A. Autistic Spectrum Disorder

Autistic spectrum disorder (ASD) is a term referring to the five pervasive developmental disorders (PDD) characterized by symptoms in three domains: 1) impairments in social interaction, 2) impairments in verbal and non-verbal communication, and 3) repetitive behaviors or interests (American Psychiatric Association, 2000). These developmental disorders are defined by the presence of behavioral symptoms. Diagnostic criteria are defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM IV-TR). The assignment of one of the five subtypes of ASD is based upon number and distribution of symptoms among the three main domains, as well as age of onset. These five disorders include autistic disorder (classic autism), Asperger syndrome, Rett syndrome, child disintegrative disorder (CDD) and pervasive developmental disorder not otherwise specified (PDD-NOS) (Muhle, et al., 2004).

The most severe form of ASD is autistic disorder, also known as classic autism. This is often the main focus of ASD research. The diagnostic criteria for classic autism as defined by the DSM IV-TR are as follows:

Patients who qualify as autistic must have at least six of the following criteria, with at least two in the first main category at least one in each remaining category:

I. Qualitative impairment in social interaction manifested by at least two of the following:

1) Impairment in nonverbal behaviors such as facial expressions, gestures and eye contact.

2) Failure to develop peer relationships appropriate for their age.

3) A lack of seeking to share his/her enjoyments, interests, or achievements with others.

4) Lack of social or emotional reciprocity, including difficulty interpreting or understanding the emotions of others.

II. Qualitative impairments in communication manifested by at least one of the following

5) A delay in, or total lack of, the development of spoken language, without a replacement with nonverbal communication like mime or gesture.

6) Stereotyped and repetitive use of language.

7) In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others.

8) Lack of make-believe or social-imitative play.

III. Restricted repetitive and stereotyped patterns of behavior, interests, and activities as manifested by at least one of the following:

9) Preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus.

10) Apparent inflexibility of adherence to specific routines or rituals.

11) Stereotyped and repetitive motor mannerisms.

12) Persistent preoccupation with parts of objects.

(American Psychiatric Association, 2000)

Symptoms of autistic disorder are observable in children at a very early age. Diagnosis usually occurs between 12 and 30 months of age, when lagging developments in language and social interactions become readily apparent (US Department of Health and Human Services, 2004). Some parents report observable symptoms in their child very early in infancy, with some noticing differences just after birth. Others report the change as being sudden, with a period of seemingly normal development followed by a regression, while still others describe plateau of social and language development.

Asperger syndrome is a higher-functioning autism in which the individual develops verbal language appropriate to age. Child disintegrative disorder (CDD) is an extremely rare disorder characterized by a period of normal development followed by dramatic regression and loss of previously acquired skills such as vocabulary and motor skills occurring between two and ten years of age. PDD-NOS is a disorder of symptoms similar to autism, but does not meet the qualifications of the other four subtypes. Rett syndrome is a relatively rare disorder caused by a single point mutation on the X chromosome, predominantly affecting females. It is the only pervasive developmental disorders subtype that has a clear and identifiable physiological cause (US Department of Health and Human Services, 2004).

Rett syndrome and CDD are extremely rare, so for the purposes of this paper the term ADS will refer mainly to autistic disorder, Asperger syndrome and PDD-NOS. These three disorders differ from one another primarily by the number and severity of symptoms. Diagnostic cut-off points between these disorders are relatively arbitrary and are defined by qualifications outlined in the DSM-IV.

ASD has become a subject of particular concern to researchers due to the dramatic increase in diagnosis in recent years. The most recent studies estimate that 1/150 children age 3-10 have an ASD (Center for Disease Control, 2007). Within a single decade the number of children diagnosed increased nearly ten-fold, from 1/2,500 –1/2,000 in the 1980's, to 1/333 – 1/166 in the 1990's (Szpir, 2006). It is debated that this dramatic increase is due primarily to increased awareness of the disorder and changing diagnostic standards, however no studies have been conducted to confirm this hypothesis (Hertz-Picciotto and Delwiche, 2009).

B. Causation

The specific causes of ASD are largely unknown. Epidemiological studies have attested genetic inheritance as the predominant cause, however no specific genes or gene combinations have been positively identified (Muhle, et al. 2004). Monozygotic (MZ) twin studies report an 80 to 90% concordance rate of ASD, and a 10% concordance of dizygotic (DZ) twins. Phenotypes of concordant twins often vary. A British study found a MZ concordance rate of 60% for classic autism, but found a 92% concordance rate when expanded to a broader ASD (Baily et al., 1995). This evidence leads us

to believe the disorder is caused by an interaction of genetic and non-genetic components. Given that the onset of the disorder occurs within the first few years of life, contributing environmental factors are likely prenatal. A few teratogens have been clearly linked to increased risk for autism. These include the chemical agents ethanol, thalidomide and valproic acid, as well as infectious agents such as the rubella virus (Arndt, et al. 2005). The effects of each of these teratogens are highly dependent on the period of prenatal development at which exposure occurred.

C. Anatomy of an autistic brain

The neurological deficits that cause the symptoms of ASD are also unknown. Multiple brain abnormalities have been found in post-mortem analysis of the patients with autism. Brain abnormalities have been noted in the brain stem, cerebellum, amygdala, hippocampus, basil ganglia, corpus callosum and cerebral cortex (US Department of Health and Human Services, 2004). These differences have been found both on the microscopic and macroscopic scale, however differences are inconsistent and widely variant among autistic patients.

D. Critical window of environmental exposure

Thalidomide is an antiemetic that was found to cause severe teratogenic effects when taken during the first trimester of pregnancy. Most notably, early prenatal exposure led to malformations in limb development causing short or even missing limbs (Rodier, et al., 1996). Exposure also caused craniofacial malformations including missing ears (both inside and

out) and/or deafness as well as increased prevalence of mental retardation and autism.

A Swedish study of thalidomide-exposed patients found that 5 out of the 86 thalidomide cases had autism (Rodier, 1996). Among the 86 available cases, only 15 had exposure on days 20-24 of gestation, however all 5 autistic cases had exposure on these days. Thus, 5/15 or 30% of those who had thalidomide exposure on days 20-24 of gestation resulted in autism. This finding is highly significant considering the prevalence rate of autism in the general population is only 0.6%. Conversely, the rate of autism for all other days of thalidomide exposure was 0%.

Such a substantial increase in autism following exposure to a teratogen during this window of development gives insight into the specific brain injury that may underlie the neurological deficits associated with autism. At 20-24 days of gestation, very little of the central nervous system has developed. The direct injury caused by the exposure could only occur in areas that were present or developing at the time of exposure. This substantially limits the area of the direct brain damaged based on developmental stage.

The critical window of gestation correlates with the period of neural tube closure and the development of the first neurons of the brainstem (Rodier, 2002). After neural tube closure, the developing brainstem divides into the basal and alar plates, giving rise to the cranial motor and sensory tracts respectively. These plates further segment into lateral sections called rhombomeres, where cells differentiate to form specific cranial nerve nuclei.

The critical time of thalidomide exposure suggests that an injury of these early developing cranial nerve nuclei may be the initial injury that led to autism.

Neurological anomalies of the five autistic patients in the study are consistent with deficits in specific cranial nerves (Rodier, 1996). Of the five patients with autism, three of the cases had Duane syndrome, caused by a failure of the abducens (MoVI) nerve that innervates the lateral muscles of the eye, four had Moebius Syndrome, caused by failure of the facial nerve (MoVII) that innervates facial muscles, and two had abnormal lacrimation, caused by a failure of the facial nerve (MoVII) innervation of the lacrimal apparatus causing mis-innervation of the neurons that normally supply the submandibular structure. Each of the autistic cases also had ear malformations and hearing deficits.

Ear malformations, eye motility problems and Moebius Syndrome have been previously associated with autism, showing these malformations are not specific to thalidomide-exposed autistic cases (Rodier 1996). All of these disorders are linked to brainstem injury. Autopsy cases of non-thalidomide exposed autistic patients also exhibit deficits in cranial nerves, however little research has been conducted to systematically investigate these deficits. One non-thalidomide autopsy case discovered a striking 97% reduction in the number of neurons in the facial (MoVII) cranial motor nucleus (Rodier 1996). This may suggest a malfunction occurred in the formation of the rhombomere from which the facial nucleus (MoVII) is derived, rhombomere 4. This converging evidence suggests that the initial

injury that leads to autism and the cranial nerve deficits may be one in the same.

The thalidomide cases indicate the brain injury leading to autism occurs very early in neurological development. Injury must occur in the areas that are present or developing at the time of exposure. Early derivatives of the brainstem are the only parts of the brain that are present so early in development. The specific injury may cause deficits in mitotic action or a disturbance in the formation of specific rhombomeres of the brainstem. Progenitor cells that give rise to the rest of the brain are also present, and injury in these cells may lead to secondary deficits in later forming brain areas. The injury must occur during the period of neural tube closure and development of the first neurons. By exposing model animals to chemical agents during this window of prenatal development, we may be able to disrupt development and create an animal model for autism.

E. Animal Model

Thalidomide does not cause the same teratogenic effect in rodents as it does primates. The drug itself is non-teratogenic and damage to the developing embryo is caused by a metabolite that is not manufactured in rodents (Rodier, 2002). Thus thalidomide is not useful for rodent animal studies. Valproic acid (VPA), an antiepileptic drug and mood stabilizer, causes teratogenic effects in rodents similar to those in humans. Prenatal exposure is associated with many of the same effects as thalidomide, including neurological dysfunctions, limb and craniofacial malformations, and an increased incidence of autism (Rodier 2002). VPA-exposed autistic

patients also exhibited malformations of the ears indicating damage during the same critical window prenatal development.

Previous rodent studies have found brainstem as well as behavioral deficits in VPA-exposed animals (Schneider and Przewlocki, 2005; Rodier et al., 1996). Morphological studies of the mouse embryo have demonstrated that acute exposure to VPA can cause a failure of neural tube closure and disorganization of cells of the neuroepithelium (Rodier, 2002). This suggests that we may be able to reproduce the neurological injuries during the period of neural tube closure found in thalidomide cases by exposing rats to VPA during this window of development. Examination of brain and behavioral deficits may allow us to explore this and develop a rat model for autism.

The critical developmental window in humans of gestational days 20-24 (G20-24) correlates with gestational days 9-11 (G9-11) in rats (Sadamatsu, et al., 2006). Neural tube closure occurs on approximately G11 and generation of the neurons of the cranial nerve motor nuclei begins immediately afterward. If exposure to VPA causes effects similar to thalidomide in humans, we would predict to find malformations in the cranial nerve nuclei of rats with exposure during the window that correlates with days G9-G11.

The thalidomide study found that prenatal exposure to thalidomide only resulted in autism when exposure occurred between G20-24. No autistic cases were found with exposure at any other time during gestation. This leads us to believe the teratogenic effects that lead to autism are time-dependent. If this is the case, we would predict malformations in the cranial nerves only when acute exposure occurs during a specific window of

gestation. Exposure on all other days would likely have little effect. Studies of rats with acute prenatal exposure to ethanol have revealed that cranial nerve deficits occur within a specific time-dependent window of vulnerability (Mooney and Miller, 2007). Prenatal exposure to ethanol is also associated with craniofacial malformations and an increased risk of autism (refs xxx Jones and Smith, 1973; Nanson, 1992), This suggests we may see time-dependent differences in animals with acute prenatal exposure to VPA. By giving animals acute exposure to VPA on different days of gestation spanning the expected critical window, we will be able to determine if deficits are specific to this period of prenatal development. A window from G9-13, allows us to examine the effects of acute exposure from just after gastrulation (G9) to the development of the facial cranial nerve neurons (G13) in order to determine a critical window of vulnerability.

Cranial nerves for our study were sampled to determine whether injury affected only neurons undergoing their final mitosis during exposure, or whether some other factor of selection was involved. To investigate this, we studied sensory cranial nerves from multiple rhombomeres to determine the presence of specific differences. We combined the present data with previously generated data on motor cranial nerve nuclei to compare the effects of VPA on both the basal and alar plates. Injury or deletion of an entire rhombomere would suggest malformations in nuclei of both plates located on the same rhombomere.

Our study focused on the determining the size and cell density of the principal sensory nucleus (PSN) and the oral and interpolar subnuclei of the

spinal trigeminal complex (SpVo, and SpVi, respectively). The PSN develops on G12-13, from the 3rd rhombomere. The SpVo develops primarily on G13-14 from the 5th rhombomere, and the SpVi develops on G14-15 out of the 7th rhombomere. By using nuclei on different rhombomeres on both plates that develop on different days we may be able to determine how the teratogen causes brainstem malformations.

We hypothesized that acute exposure during the period of neural tube closure will lead to deficits in cranial nerve nuclei in a time-and site-dependent manner.

II. Materials and Methods:

A. Animals

Long Evans rats were mated, and pregnancy was determined by the presence of a vaginal plug. The day the plug was found was labeled as the first day of gestation (G1). Sodium valproate was dissolved in saline and the pH was adjusted to 7.3. Each treated dam received a single intraperitoneal (i.p.) injection of 350mg/kg body weight of sodium valproate in solution on a single day of gestation from G9 through G13 inclusive. Control dams were given a single intraperitoneal injection of an equivalent volume of saline. The day of birth was designated as postnatal day one (P1). On postnatal day 30 male offspring were taken from each litter and anesthetized with an intraperitoneal injection of a 100 mg/kg ketamine and 10 mg/kg xylazine mix. The animal was then perfused transcardially with a solution of 4.0% paraformaldehyde in 0.10 M phosphate buffer (PB). The brains were

removed, post-fixed in a fixative for 4 hours at room temperature, washed in PB and stored in fresh PB at 4 °C.

Brainstems were isolated and dehydrated through increasing concentrations of ethanol, cleared with butanol, then infiltrated with Paraplast Plus paraffin. The paraffin embedded tissue was then cut into 5μm sections. Half of the samples for each treatment day were cut into sagittal sections while the remaining half was cut into horizontal sections. The 5μm sections divided the entire brainstem, with one in every 10 sections put on a slide and taken for analysis. These sections were de-paraffinized in xylene, rehydrated, stained with cresyl violet then dehydrated through increasing concentrations of ethanol before being coverslipped.

B. Anatomic Studies

Stereological methods were used to estimate the volume and cell density of two cranial nerve motor nuclei: the facial (VII) and the trigeminal (V), as well as three sensory nuclei: the principal sensory nuclei (PSN) and the oral and interpolar subnuclei of the spinal trigeminal nuclear complex (SpVo and SpVi respectively). Estimates of the volume of the cranial nerve nuclei were determined by using the Cavalieri estimator (Jenson et al., 1987; Mooney and Miller, 2007). In each section in which the cranial nerve nucleus was visible the cross sectional area was measured using the Bioquant Image Analysis System (R&M Biometrics, Nashville TN). The borders of each nucleus were determined based on cytoarchitectonic criteria. The neuron cell bodies of the each of the two cranial motor nuclei were much larger and more densely packed than surrounding cells. The principal sensory was identified based on

its disk –shape appearance and its characteristic very small and densely packed neurons. The oral nucleus was identified based on its hourglass structure and medium-size neurons organized in striations. The interpolaris was identified based on its relatively small medium packed neurons and characteristic shape.

The total volume was determined by summing cross sectional areas and multiplying by the section thickness (5μm) times the inverse of the section frequency (1/10). This method is illustrated in the formula below:

$$V_T = \Sigma A_s * t/f$$

where ΣA_s is the sum of the cross sectional areas, t is the section thickness (5μm), and f is the frequency of sections in the series (1/10).

The cell density of the nuclei was determined using the Smolen correction of Abercrombie's estimator (Abercrombie, 1946; Smolen et al., 1983; Miller and Muller 1989). The cranial nerve nuclei had been counted by a student previously working in the lab. For the cranial nerve motor nuclei, the number of motor neurons with visible nucleoli in a 400 x 400 μm area at x 10 magnification were counted for each section of the brainstem in which the nerve nuclei was visible. Only neurons fully within this box, or touching one of two adjacent sides of the box were counted. Neurons touching the remaining two sides were excluded from counts. The sensory nuclei consist of much smaller and more densely packed neurons and thus were estimated using same procedure with a 100 x 100 box at a magnification of x 40. The

diameter was measured for each of the counted cells. The neuronal density was calculated using the following formula:

$$N_v = n * t \ / (t + D_m - 2k)$$

Where *Nv* is the neuronal cell density, *n* is the total number of neurons counted in the boxes of the nuclei sections, *t* is the section thickness (5μm), D_m is the mean maximal diameter of the cell bodies, and *k* was the diameter of the smallest recognizable cap of a cut cell nucleus.

Estimates of the volume of the nerve nuclei were multiplied by the estimates for cell density in order to approximate the total number of neurons within the nucleus.

C. Statistical Analysis

The mean ± standard error of the mean was calculated for the volume, neuronal cell density, and neuronal number for each nucleus of each treatment day. These numbers were subject to a one-way ANOVA, to determine on overall effect of treatment. Where significant differences were found, a *post-hoc* Tukey test was performed. Significance was set at $p = 0.05$.

III. Results

All five nuclei examined were identifiable in the cresyl violet stained sections. There were no gross differences in appearance of nuclei among treatment groups. Analysis of Variance (ANOVA) for the sensory nuclei are located in Tables 1-3 in the appendix.

<u>Principal Sensory Nucleus of the Trigeminal Nerve</u>

The volume of the principal sensory nucleus of the trigeminal nerve exhibited significant differences among treatment groups ($p < .001$). A Tukey post-hoc analysis found volume to be significantly greater on G13 than in control-treated animals ($p < .05$). No significant differences from controls were found in the neuronal cell density or total number of neurons (Figure 1).

Spinal Trigeminal Nucleus, Oral portion

The volume of the oral subnuclei of the spinal trigeminal was not significantly different compared to controls. The neuronal cell density was also did not significantly differ. The total number of neurons however, was a significantly decreased in animals exposed on G13 (Figure 2).

Spinal Trigeminal Nucleus, Interpolar portions

The volume of the interpolar subnuclei of the spinal trigeminal was not significantly affected by treatment. There was a significant decrease in neuronal cell density with animals exposed on G13. The total number of neurons also significantly decreased with exposure on G13 (Figure 3).

IV. Discussion

A. Summary of Data

The total neuronal number of both the oral and interpolar subnuclei of the spinal trigeminal significantly decreased following exposure to VPA on G13. This time correlates with the peak neuronal generation of the oral subnuclei of the spinal trigeminal, and just before peak neuronal generation of the interpolar subnuclei of the spinal trigeminal. Decreased total neuronal number with exposure during neuronogenesis of these nuclei suggests VPA

exposure may affect cell proliferation. The oral and interpolar subnuclei of the spinal trigeminal derive from different rhombomeres, suggesting the mechanism that leads to deficits is not selective for a single rhombomere.

No effects in total neuronal number were seen for the principal sensory nucleus (PSN). This nucleus reaches peak neuronal generation at G12-13, however, no deficit were seen at G13. This suggests that the teratogen does not merely cause deficits in all neurons undergoing neurogenesis at the time of exposure. The teratogen must have an alternate means of selection in order to affect the oral and interpolar subnuclei of the trigeminal while sparing the principal sensory nucleus. Perhaps the teratogen does not effect rhombomeres so far rostal, and only affects the more caudal sections.

The principal sensory nucleus only exhibited significant differences from the control on one occasion, in which the volume of the nuclei was significantly greater with exposure on G13. This is the same day in which we saw a significant decrease in the total neuronal number in the other two sensory nuclei (SpVo and SpVi). Exposure to VPA may have caused an inverse effect in the PSN, leading to an increased volume. The total number of neurons was not affected so the cause of the increased volume was not due to increased neuron proliferation. An increase in the production of fibers or glial cells within the nucleus may have accounted for the volume increase.

To check for differences between effects in the alar and basal plates, we compared the sensory data to previously collected motor nuclei data (Figures 4 and 5). Neurons of the facial motor nucleus (MoVII) develop on G13, out of the 5th rhombomere. Neurons of the trigeminal motor nucleus

(Mo V) form on G12, from the 3rd rhombomere. Analysis of MoV found significantly smaller volumes in all treatment groups when compared to the controls. We also found significantly greater cell density in all treatment groups when compared to controls. This data combined to yield no significant differences in total neuronal number between control and treatment groups.

Much like the results of MoV, inverse differences in volume and neuronal cell density yielded no significant differences in total neuronal number the facial (VII) nuclei. Significantly smaller volumes were found for treatment days G9, G11, and G13, and significantly lower neuronal cell density was found for the same three treatment days. These results again lead to no significant differences in the total neuronal cell number.

It is difficult to draw conclusions on VPA affect on the two motor nuclei. The control data for the motor nuclei had not been conducted so control data used for analysis was taken from a study on time-dependent effects of ethanol exposure on cranial nerves (Mooney and Miller, 2007). While this was control data and thus should have little effect on the analysis, differences in inter-rater counting techniques may have affected our results. From the data it is difficult to draw specific conclusions about the effect of treatments when compared to controls, however it appears the treatment had little effect on the total neuronal number on the motor cranial nerves that were examined, and no time-dependent differences were revealed. This suggests that the basal plate was largely protected from VPA-induced toxicity.

B. Literature Comparison

A study of time-dependent differences in rat cranial nerves with acute exposure to ethanol found a significant decrease in total neuronal number in the principal sensory nucleus, the trigeminal motor nucleus (MoV), and the facial nucleus (MoVII) after exposure on G12 and G13 (Mooney and Miller, 2007). No significant differences were found in the SpVo or SpVi. These findings do not parallel the effects seen with prenatal VPA, suggesting that ethanol and VPA may affect different areas of the brain selectively. That said, exposure to a teratogen on G13 has an effect on both cranial motor and sensory nuclei, and thus, is a window of vulnerability for cranial nerve nuclei.. This may be due to the window of neuronogenesis, or an alternate factor arising during this time of development.

A similar study on the prenatal effects of VPA on cranial nerves found no effect in the facial nuclei following acute exposure on rats on days G11.5, G12 and G 12.5 (note that G12.5 in this study = G13 in our study; Rodier, 1996). This is consistent with the data we found, and suggests that acute VPA exposure does not affect the facial nucleus. The Rodier study did not give data for nuclei volume and neuronal density.

The same study by Rodier did not parallel our results for MoV. They reported a significant decrease in neuronal number of the trigeminal (MoV) with exposure on G11.5 and G12, while our study did not find any significant changes after treatment on G12 (equivalent to their G11.5). It is unclear why we did not find similar results in the trigeminal (MoV). Difference in results

may be due to experimental techniques. More studies need to be conducted in order to determine whether the trigeminal neuronal number is affected.

Our data did not find any effects in neuronal number of the motor nuclei, but did find decreased neuronal number in two sensory nuclei. This suggests the alar plate may be more susceptible to deficits caused by VPA exposure. No differences in the PSN show that not all nuclei of the alar plate undergoing neuronogenesis are affected, suggesting other factors of selection must be involved.

The oral and interpolar subnuclei of the spinal trigeminal complex exhibited a significant decrease in neuronal number following exposure to VPA on G13. This data suggests that deficits caused by VPA exposure may target more than one rhombomere as both the oral and interpolar subnuclei are affected.

Convergent data with other VPA studies and ethanol studies suggest teratogen exposure may cause greater deficits in the cranial nerves when exposed on G13 than on surrounding days. Exposure to VPA in rats on G13 appears to be a critical window, leading to deficits in the oral and interpolar subnuclei of the spinal trigeminal.

C. Future Research

More motor nuclei must be examined to determine if the basal plate is at all affected by VPA exposure. Studies should also retest the trigeminal (MoV) in order to determine why we did not see differences in neuronal number while the Rodier study did find differences. We should also examine

sensory nuclei deriving from other rhombomers, to determine where an affect is evident. This will help us understand why effects were seen in the two subnuclei of the trigeminal (SpVo and SpVi), but not seen in the PSN.

Research should also look into brain deficits in higher-functioning parts of the brain in VPA exposed rats, such as the amygdala and the cerebral cortex, in order to determine the presence of secondary effects caused by acute exposure to VPA during the critical window of exposure (which appears to be G13). Studies may then expand to examine behavioral deficits in order to further develop VPA exposed rats as an animal model for autism.

The VPA model for autism has serious potential for aiding in autism treatment and research. Discovery of a possible window of vulnerability leads to significant insight on the parts of the brain that may be affected, namely the brainstem. Research can expand beyond the cranial nerve nuclei to determine what parts of the mature brain are damaged by teratogenic exposure during neural tube closure and generation of the first neurons. We can also research higher functioning parts of the mature brain that may have been secondary effects of damage caused in development of the brainstem. While the initial damage that leads to autism a may occur in the brainstem, these secondary affects may lead to symptoms observed in autism.

Any animal model of autism is inherently limited due to the diagnostic standard, based on communication, social interaction and emotional reciprocity which cannot be seen in animal models to the same extent as humans. A number of studies have developed to test for autistic traits,

particularly with social interaction and repetitive behaviors (Crawley, 2007). These tests have yielded promising results for the VPA exposed rat as a model for autism. Behavioral tests have found VPA exposed rats to exhibit autistic traits such as increased repetitive behaviors and latency to social behavior (Schneider, 2005). Communication deficits have also been examined by testing ultrasonic vocalization patterns (Crawley, 2007). The formation of refined behavioral tests may allow us to further explore how this model may parallel the symptoms of autism. Further research must be conducted using VPA exposure and behavioral tests in tandem to determine connections between behavioral and neurological deficits observed. We must combine this with more autopsy cases in order to determine the presence of consistent neurological deficit in autistic patients.

Development of a rat model for autism will help researchers understand the disorder, as well as provide a mechanism for exploring treatments. Tests have already shown some success in altering behavioral deficits in VPA exposed rats through particular therapies and enriched environments. (Schneider, et al., 2006). The development of an animal model will be particularly useful in creating and testing neuropharmacological drugs to treat autism in the future.

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VI. Appendix

Table 1

ANOVA Analyses of Nuclear Volume

Cranial Nerve Nuclei	F – statistic	Degrees of freedom	P value
PSN	9.555	5	<0.001*
SpVo	5.784	5	0.002*
SpVi	3.000	5	0.037*
Mo V	14.455	5	<0.001*
Mo VII	11.066	5	<0.001*

Table 2

ANOVA Analyses of Neuronal Cell Density

Cranial Nerve Nuclei	F - statistic	Degrees of freedom	P value
PSN	5.612	5	0.002*
SpVo	6.618	5	<0.001*
SpVi	6.432	5	<0.001*
Mo V	12.685	5	<0.001*
Mo VII	11.160	5	<0.001*

Table 3

ANOVA Analyses of Total Neuronal Number

Cranial Nerve Nuclei	F - statistic	Degrees of freedom	P value
PSN	1.725	5	0.173
SpVo	4.762	5	0.006*
SpVi	6.443	5	0.001*
Mo V	1.453	5	0.249
Mo VII	1.999	5	0.125

* Statistically significant difference

Figure 1

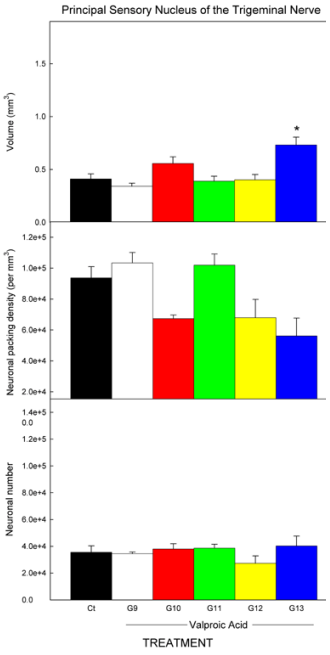


Figure 2

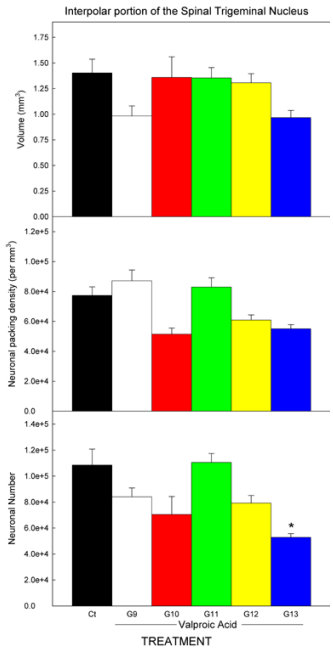


Figure 3

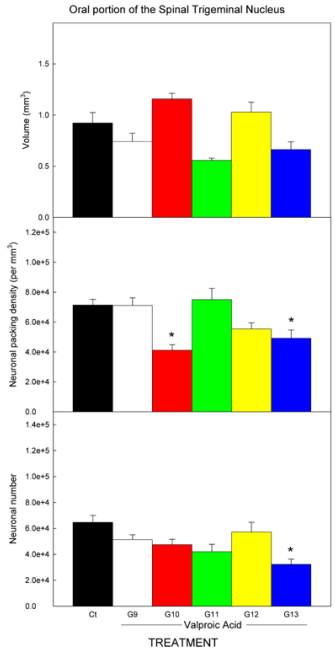


Figure 4

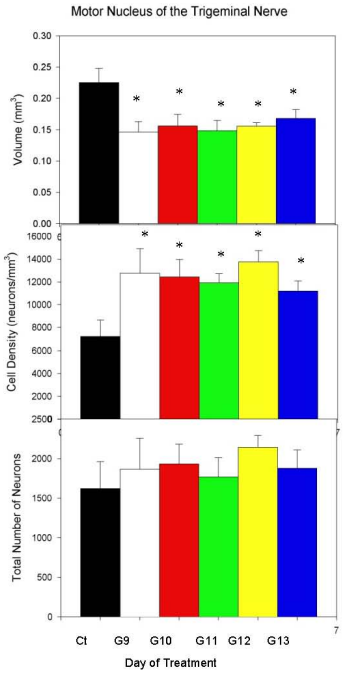
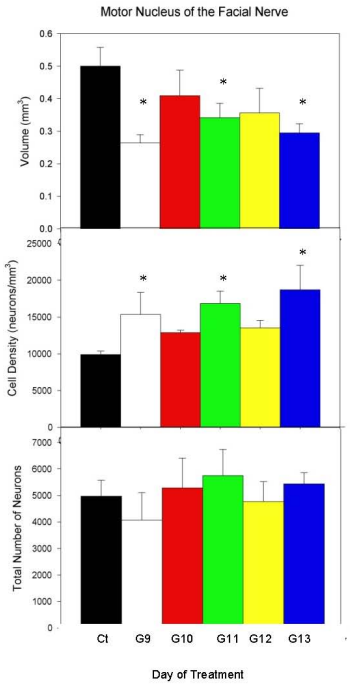


Figure 5



*Statistically significant difference from control